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Toxicology and Applied Pharmacology 207 (2005) S96 – S102

Toxicology and Applied Pharmacology

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Review

Roles of thioredoxin in nitric oxide-dependent preconditioning-induced tolerance against MPTP neurotoxin

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Received 11 July 2004; revised 2 March 2005; accepted 31 March 2005
Available online 7 July 2005

Abstract

Hormesis, a stress tolerance, can be induced by ischemic preconditioning stress. In addition to preconditioning, it may be induced by other means, such as gas anesthetics. Preconditioning mechanisms, which may be mediated by reprogramming survival genes and proteins, are obscure. A known neurotoxicant, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), causes less neurotoxicity in the mice that are preconditioned. Pharmacological evidences suggest that the signaling pathway of $^{\bullet}$NO–cGMP–PKG (protein kinase G) may mediate preconditioning phenomenon. We developed a human SH-SY5Y cell model for investigating $^{\bullet}$NO-mediated signaling pathway, gene regulation, and protein expression following a sublethal preconditioning stress caused by a brief 2-h serum deprivation. Preconditioned human SH-SY5Y cells are more resistant against severe oxidative stress and apoptosis caused by lethal serum deprivation and 1-mehtyl-4-phenylpyridinium (MPP^+). Both sublethal and lethal oxidative stress caused by serum withdrawal increased neuronal nitric oxide synthase (nNOS/NOS1) expression and $^{\bullet}$NO levels to a similar extent. In addition to free radical scavengers, inhibition of nNOS, guanylyl cyclase, and PKG blocks hormesis induced by preconditioning. *S*-nitrosothiols and 6-Br-cGMP produce a cytoprotection mimicking the action of preconditioning tolerance. There are two distinct cGMP-mediated survival pathways: (i) the up-regulation of a redox protein thioredoxin (Trx) for elevating mitochondrial levels of antioxidant protein Mn superoxide dismutase (MnSOD) and antiapoptotic protein Bcl-2, and (ii) the activation of mitochondrial ATP-sensitive potassium channels [K(ATP)]. Preconditioning induction of Trx increased tolerance against MPP^+, which was blocked by Trx mRNA antisense oligonucleotide and Trx reductase inhibitor. It is concluded that Trx plays a pivotal role in $^{\bullet}$NO-dependent preconditioning hormesis against MPTP/MPP^+.

Keywords: Adaptation; Antiapoptotic protein Bcl-2; Cyclic GMP; Hormesis; Mitochondria; Mn superoxide dismutase; 1-Methyl-4-phenylpyridinium (MPP^+); ATP-sensitive potassium channel

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doi:10.1016/j.taap.2005.03.020

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Introduction

Selective and non-selective neurotoxicity caused by MPTP/MPP$^+$

MPTP is a man-made neurotoxin which is converted to toxic metabolites such as 1-methyl-4-phenylpyridinium (MPP^+). MPP^+ enters brain monoaminergic neurons and causes a selective destruction of the A9 nigrostriatal dopaminergic neurons in monkeys and humans at low milligram doses (Burns et al., 1983). At 1.5 mg/kg intravenous (iv) dose of MPTP, MPP^+ is taken up by brain dopaminergic neurons but preferentially it causes retrograde degeneration of the pigmented A9 nigral neurons only and spares non-pigmented brain dopamine neurons (A10, A12 and A 16) (Chiueh et al., 1985). It is worthy noting that the pigmented A9 nigral neurons contain relatively high levels of non-heme iron complexes (Chiueh, 2001). We proposed that sustained dopamine release caused by MPP^+ could increase auto-oxidation of dopamine in the presence of iron complexes and oxygen (Chiueh et al., 1994). Dopamine oxidation generates hydroxyl radicals, lipid peroxidation, oxidative stress and retrograde degeneration predominantly in iron-rich A9 nigral neurons when the cellular antioxidative defense system is compromised by sustained high oxidant stress (Chiueh, 2001; Chiueh and Rauhala, 1998). In fact, ferrous citrate complexes are as toxic as MPP^+ in causing nigral loss in vivo indicating that the mixture of iron, oxygen and dopamine is highly neurotoxic and selective to pigmented A9 nigral neurons (Sun et al., 1988; Sziraki et al., 1998). Unexpectedly, the highest concentration of MPP^+ is located in monkey's noradrenergic cell bodies (locus caeruleus and adrenal medulla) where no significant neurotoxicity is found (Markey et al., 1984). When experimenting with high concentrations in non-dopaminergic cells, MPP^+ causes oxidative damage and necrotic death in cells and neurons since it not only complexes with iron to generate cytotoxic-free radicals but also inhibits mitochondrial complex I and energy supply (Andoh et al., 2002a; Kotake and Ohta, 2003).

In the rodents MPTP (>100 mg/kg) causes a serotonin syndrome and a reversible acute dopamine depletion rather than chronic nigrostriatal neurodegeneration (Chiueh et al., 1984). The administration of low doses of MPTP in rhesus monkeys (<1.5 mg/kg, iv) creates an ideal primate model of parkinsonism reflected by a selective nigrostriatal degeneration, dopamine depletion and nigral loss. This MPTP-induced primate model, but not rodent model, can be used for screening of new therapeutics, testing of brain dopamine imaging ligands, and transplantation efficacy using dopamine producing neurons and stem cells (Chiueh, 1988). However, unpublished information suggests that adaptive hormesis (e.g., concentrations of toxic substances below the amount that cause toxicity will cause tolerance) may be developed in monkeys when much lower doses of MPTP are chronically administered (C. Freed, personal communications). This adaptation phenomenon could explain why only a few parkinsonian cases developed among approximately 200 MPTP abusers. For elucidating this hormesis mechanism, we employed a human neuroblastoma SH-SY5Y cells to examine preconditioning tolerance in preventing or decreasing MPP^+-induced neurotoxicity (Andoh et al., 2000). We also investigated preconditioning-induced survival proteins and to understand which gene or protein elicits preconditioning-induced adaptive neuroprotection (Andoh et al., 2002a). We focused on cGMP-dependent reprogramming of early genes in the nucleus and survival proteins in the mitochondria of preconditioned human neuroblastoma cells since free radicals often affect nuclear DNA and mitochondrial proteins (Andoh et al., 2002b, 2003).

Roles of free radicals in initiating and maintaining preconditioning adaptation

In the cardiovascular system, brief episodes of ischemia cause new protein synthesis and subsequently protect cells from prolonged period of lethal ischemia insult, which is now recognized as a new research field of preconditioning phenomenon. The exact preconditioning mechanism underlying adaptive neuroprotection is largely unclear, which remains to be elucidated. Increasing evidence suggest that free radicals may be involved not only in the trigger of preconditioning phenomenon in the cell nucleus but also the maintenance of adaptive expression of survival proteins, especially in the mitochondria (Andoh et al., 2000; Nakano et al., 2000). Most preconditioning procedures are blocked by free radical scavengers, and some of delayed adaptive cytoprotections are prevented by inhibition of subtypes of nitric oxide synthase (NOS) depending on the types of cells studied (Andoh et al., 2000; Centeno et al., 1999; Gidday et al., 1999; Horimoto et al., 2000; Keynes and Garthwaite, 2004; Xi et al., 2002). Our pilot in vitro study indicates that serum withdrawal increases first the generation of hydroxyl radicals ($^\bullet$OH) and a delayed increase of nitric oxide ($^\bullet$NO) (Andoh et al., 2000).

In myocardial preparations, preconditioning activation of eNOS and associated $^\bullet$NO–cGMP–PKG (protein kinase G) pathway in the cardiovascular system can phospho-activate ATP-sensitive potassium channels [K(ATP)] in the heart for cardioprotection (Garlid et al., 2003; Han et al., 2002; Horimoto et al., 2000; Ockaili et al., 1999; Zaugg et al., 2002). It is also known that a similar preconditioning neuroprotective mechanism via activation of mitochondrial K(ATP) channels can be observed in the central nervous system (Kis et al., 2004). Ischemic preconditioning-induced